American Journal of Medical Genetics 63:335–339 (1996)

Leiomyoma of Uterus in a Patient With Ring Chromosome 12: Case Presentation and Literature Review

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We report on a 30-year-old woman with de novo ring chromosome 12 mosaicism, 46,XX, r(12)(p13.3q24.3)/46,XX. In addition to the clinical manifestations generally observed in "ring syndrome" cases such as growth retardation, short stature, microcephaly, and mental deficiency, she had a broad nasal bridge, micrognathia with overbite, underdeveloped breasts, mild dorsal scoliosis, clinodactyly of the fifth fingers with a single interdigital crease, symphalangism of thumbs, tapering fingers, mild cutaneous syndactyly between the second and third toes, multiple café-au-lait spots, sebaceous acne on the face and back, and mild dystrophic toenails. She developed a large, pedunculated uterine leiomyoma at age 28 years. To our knowledge, uterine leiomyoma in association with r(12) has not been reported previously. However, a gain of chromosome 12 and translocations involving 12q14-15 have been described.

KEY WORDS: ring chromosome 12, uterine leiomyoma, ring syndrome

INTRODUCTION

Formation of a ring chromosome is associated with the loss of genetic material. Diversity in clinical manifestations among patients with an apparently similar ring chromosome is probably due to subtle differences in the breakpoints, making it difficult to establish a genotype-phenotype correlation [Kosztolányi et al., 1987]. It appears that ring chromosome instability during the cell cycle also contributes to this phenotypic diversity, although the mechanism is not completely understood.

Here we present a patient with r(12), severe growth retardation, and mental deficiency, who later developed a uterine leiomyoma. We discuss the possibility of ring chromosome instability leading to her clinical manifestations.

CLINICAL PRESENTATION

The proposita is 30 years old. She was born to a 23-year-old, G1 mother and a 29-year-old father by normal vaginal delivery at term. Gestational history was unremarkable except for an episode of pharyngitis associated with high fever during the first trimester. Birthweight was 2.24 kg; and length was 38 cm. During infancy she had intermittent diarrhea. She sat at 9 months, stood and walked with support at 2 years, and spoke her first words at 1½ years. At age 5, she was noticed to be short; a chromosome analysis at that time showed ring chromosome 12 mosaicism. Parental chromosomes were normal. She attended a regular school, but reportedly regressed in mental status following measles pneumonia at age 8, and subsequently required special education.

She developed ascites at age 28. The T4, TSH, and CA-125 levels were normal. Abdominal and pelvic ultrasound and CT scan studies did not indicate the presence of any mass or lymphadenopathy. Upper GI series and endoscopy showed dilated stomach and rapid small intestinal transit. Culture of ascitic fluid for *Mycobacterium tuberculosis* was negative. The patient was subsequently referred to our center. A repeat chromosome analysis confirmed the previous findings of r(12) mosaicism. Eventually laparotomy was performed, and a large, pedunculated uterine mass removed. Histopathology demonstrated leiomyoma of uterus.

On examination, the patient was alert and cooperative, although noncommunicative. Her weight was 23 kg (<3rd centile, equal to the 50th centile for a 7-year-old girl), height was 126 cm (<3rd centile, equal to the 50th centile for an 8-year-old girl), and head circumference was 48.5 cm (<3rd centile, equal to the 50th centile for a 2½ year-old girl). She had epicanthic folds, long eyelashes, broad nasal bridge, and moderate to severe micrognathia with overbite (Figs. 1–3). The thorax was symmetrical with underdeveloped breasts, well-developed areolae, and normal axillary hair. Lungs

Received for publication August 26, 1994; revision received November 20, 1995.

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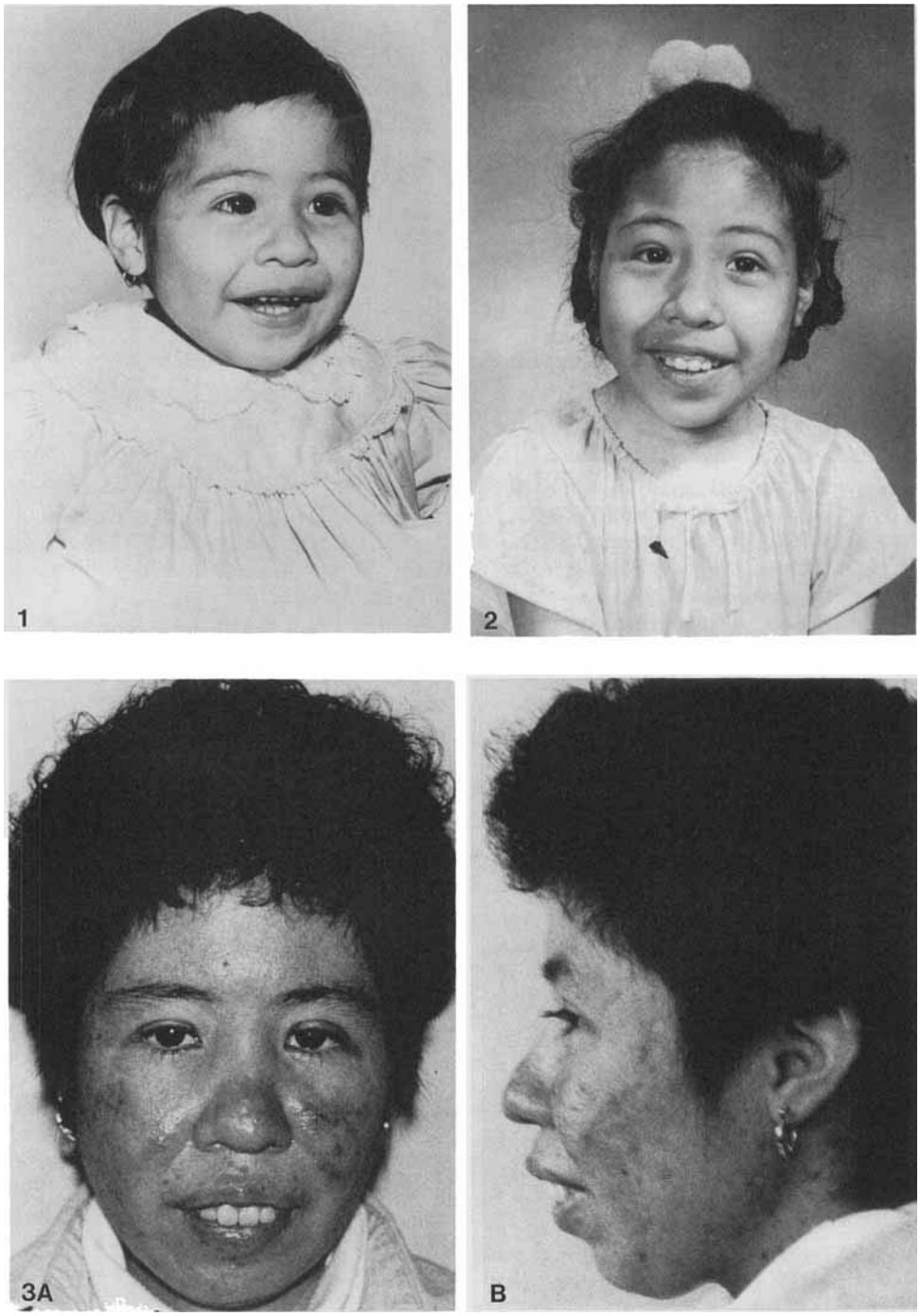

Fig. 1. Patient at age 3 years.
Fig. 2. Patient at age 12 years.
Fig. 3. Facial appearance of patient at time of examination, age 30 years: frontal **(A)**, lateral **(B)**. Note the change of facial appearance as age advances.

and heart were normal on auscultations. The spleen was palpable. The lower region of the abdomen was protuberant with a dullness on percussion; genitalia were normal. There was a mild dorsal scoliosis to the left. Hands were small with tapering fingers, symphalangism of thumbs, and a mild clinodactyly of the fifth fingers with a single interdigital crease. Her feet showed mild cutaneous syndactyly between the second and third toes. Skin showed multiple café-au-lait spots of varying size and color. No axillary freckling was observed. She had multiple acnelike lesions on her face and back. The toenails were mildly dystrophic. Muscle mass was reduced, but the muscle tone was normal. Neurological findings were unremarkable.

The patient has two brothers, 17 and 27 years old. Her mother, two brothers, and several maternal first-degree relatives have multiple café-au-lait spots, but no other sign of neurofibromatosis type I (NF-1).

MATERIALS AND METHODS

GTG-banded chromosome analysis of peripheral blood lymphocytes was performed by conventional methods. Genomic DNA from blood leukocytes and paraffin-embedded leiomyoma tissue were prepared according to standard protocols [Lahiri and Nurnberger, 1991]. The five markers within the telomeric regions of the short and long arms of chromosome 12, D12S93, D12S79, D12S97, D12S352, and D12S357 were kindly provided by Dr. K.T. Montgomery (Albert Einstein College of Medicine, Bronx, NY).

These markers were used in single polymerase-chain reactions (PCR). The reactions were performed in a total volume of 20 μl, using 100–200 ng of genomic DNA from the patient's blood cells, her leiomyoma tissue, and a control blood sample. Reaction mixtures were 200 μM in each dNTP; 1.5 mM in $MgCl_2$; and 1 μM in each primer pair, and included 1 U Taq DNA polymerase (Promega, Madison, WI), as well as the DNA in the quantities mentioned above (for all but the blank). Initial denaturation was at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. Aliquots of the reactions were mixed with tracking dye and electrophoresed on either a 2% agarose (Bio-Rad Laboratories, Hercules, CA), or a 6% horizontal polyacrylamide (Sigma Chemical Co., St. Louis, MO) gel. The gels were stained with ethidium bromide, visualized under ultraviolet light, and photographed.

RESULTS

In peripheral blood she had a de novo 46,XX,r(12)(p13.3q24.3)/46,XX karyotype; 12 of 38 cells (32%) had a 46,XX chromosome complement.

PCR with the dinucleotide repeat marker D12S93, located in the 12p13.2-pter region, produced two bands (each representing a separate allele of chromosome 12) in the patient's blood-cell, leiomyoma, and control DNA samples (Fig. 4). Thus the breakpoint was established distal to p13.2. Reactions with marker D12S79, located at 12q24.1-qter, produced two bands in all DNA samples, confirming the cytogenetic breakpoint as distal to q24.1. Markers D12S97, located at 12q24.3 (Fig. 4), and D12S357, located at 12qter, were not informative, forming one band in all samples. However, the dinucleotide repeat marker D12S352, located at 12pter, was informative: agarose gels electrophoresed with these reaction products showed the presence of one band in patient leiomyoma and two bands in both patient leukocytes and control leukocytes (Fig. 5). The markers, their loci, and the heterozygosity values of the five markers are shown in Table I.

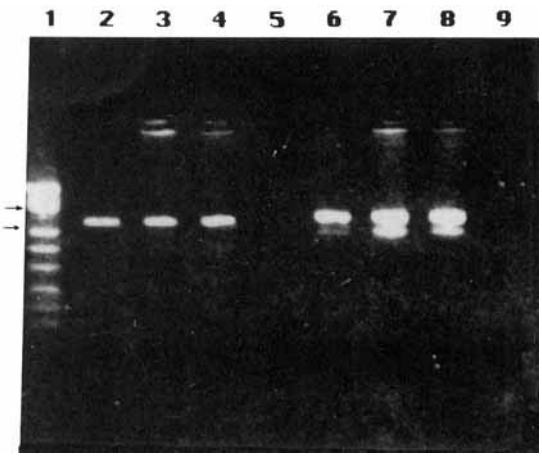

Fig. 4. This figure represents the results of two separate experiments. Lanes 2–4: Marker D12S97 (not informative). Lanes 6–8: Marker D12S93 (informative), showing two bands in all DNA samples, confirming the cytogenetic breakpoint in the ring chromosome 12 as distal to p13.2. Top arrow: 331 bp; bottom arrow: 242 bp. Marker D12S97 generates fragments of 265–279 bp, whereas D12S93 reaction products are 271–291 bp. **Lane 1:** Molecular-weight marker (pUC18 digested with Msp I). **Lane 2:** Leiomyoma DNA. **Lane 3:** Patient leukocyte DNA. **Lane 4:** Control blood DNA, **Lane 5:** Blank: no DNA in PCR reaction. **Lane 6:** Leiomyoma DNA. **Lane 7:** Patient leukocyte DNA. **Lane 8:** Control blood DNA. **Lane 9:** Blank: no DNA in PCR reaction.

DISCUSSION

Our patient shares many phenotypic manifestations with other reported cases of ring chromosome 12 [Park et al., 1988; Scribanu et al., 1980] (Table II), including growth failure, short stature, microcephaly, café-au-lait spots, clinodactyly of 5th fingers, developmental delay, speech deficiency, and mental retardation. Hypothyroidism and elevated antithyroid antibodies have also been reported [Park et al., 1988].

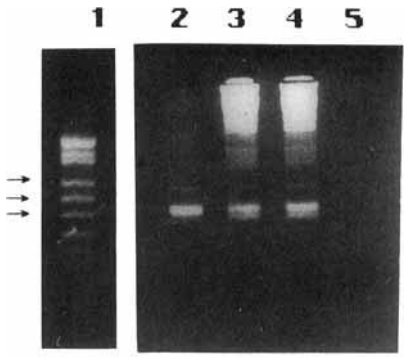

Fig. 5. Marker D12S352 (informative) showing the presence of 1 band in the leiomyoma DNA and two bands in the patient's leukocytes as well as in the control blood cells, establishing loss of heterozygosity in the tumor and suggesting the clonal development of the leiomyoma from the ring chromosome 12 cell line. Top arrow: 242 base pairs (bp); middle arrow, 190 bp; bottom arrow, 147 bp. DNA fragments synthesized by PCR reactions with this marker are 148–162 bp. **Lane 1:** Molecular-weight marker (pUC18 digested with Msp I). **Lane 2:** Leiomyoma DNA. **Lane 3:** Patient leukocyte DNA. **Lane 4:** Control blood DNA. **Lane 5:** Blank: no DNA in PCR reaction.

TABLE I. Dinucleotide Repeat Polymorphisms in the Telomeric Regions of Chromosome 12

Marker symbol	Locus	Map	Product size (bp)[a]	# allele	Heterozygosity [Gyapay et al., 1990]
AFM205Ve5	D12S93	12p13.2-pter	271–291	8	0.81
AFM067Yc5	D12S79	12q24.1-qter	155–179	11	0.87
AFM2102d6	D12S97	12q22-qter	265–279	7	0.68
AFM303Xd9	D12S352	12pter	148–164	8	0.73
AFM310Vd5	D12S357	12qter	193–225	11	0.85

[a] bp = base pair(s).

Café-au-lait spots (CALS) have been reported in patients with ring chromosome 12. CALS have also been reported as an isolated trait with an autosomal dominant pattern of inheritance, and as part of other disorders, such as tuberous sclerosis (TSC) and NF-1. Further workup, including brain CT scan, ophthalmological and audiological evaluation, echocardiogram, and Wood's lamp examination, did not support the possibility of TSC or NF-1 in this patient. The cause of the CALS in this patient, her mother and brothers, and maternal first-degree relatives remains to be determined.

The phenotypic diversity among patients with an apparently similar ring chromosome is probably due to the subtle differences in the ring chromosome breakpoints. Thus establishing a genotype-phenotype correlation is difficult. Côté et al. [1981] proposed the existence of a peculiar phenotype of "ring syndrome" in patients with an autosomal ring. The single major manifestation of this phenotype is severe somatic retardation without an apparent organic, biochemical, or endocrine cause. The formation of a ring chromosome, in some instances, is associated with the loss of some genetic material. However, Pezzolo et al. [1993] showed the presence of telomeric and subtelomeric sequences at the fusion points of ring chromosomes by fluorescence in situ hybridization. Therefore, it appears that the ring syndrome is not a consequence of the loss of genetic material, but rather the result of ring instability [Côté et al., 1981; Kosztolányi et al., 1987; Pezzolo et al., 1993].

A striking finding in our patient is the uterine leiomyoma. A gain of chromosome 12 and translocations involving 12q14-15 have been reported in uterine leiomyoma tissue. In a study of 34 uterine leiomyomas, Heim et al. [1988] found an apparently identical reciprocal translocation, t(12;14)(q14-15;q23-24) in the neoplastic tissues of four patients. Vanni and Lecca [1988] found a t(X;12)(p22.3;q15) in a single uterine leiomyoma and suggested that the 12q14-q15 region may have one or more genes involved in neoplastic proliferation. Nilbert and Heim [1990] reviewed 104 reported leiomyomas with karyotypic aberrations. Four major subgroups, including 6p aberrations; del(7)(q21.2q31.2); +12; and t(12;14)(q14-15;q23-24) were identified. In one-third of the cases, a ring chromosome was identified, often along with one of the aberrations listed above, but, except for one r(1)—as the sole rearrangement—most of the ring material was unidentifiable.

In our patient, the leiomyoma tissues were not submitted for cytogenetic analysis. However, we suspected that the r(12) cell line may have initiated the tumor formation. To examine this possibility, DNA analysis of available formalin-embedded leiomyoma tissue was performed (Figs. 4, 5). Examination of the copy numbers of the informative marker D12S352, located in the pter region, showed loss of heterozygosity (LOH) in the leiomyoma (Fig. 5). This finding is suggestive of the replacement of the diploid cell line by the r(12) cell line in the tumor tissue. Further chromosome abnormalities may have occurred due to ring chromosome instability. For instance, sister chromatid exchange within a ring produces dicentric or interlocked rings. These may lead to anaphase lag, hence aneuploidy, formation of micronuclei, pulverized chromosomes, and rings of different sizes, resulting in cells without a ring or with

TABLE II. Comparison of Cytogenetics and Phenotype of Two Previously Reported Cases and the Present Case

	Scribanu et al.	Park et al.	Present case
Age at time of reporting	13 months	19 years	30 years
Sex	Female	Male	Female
Breakpoints of r(12)	(p13q24)	(p13.3q24.3)	(p13.3q24.3)
Growth retardation	++	++	++
Multiple CALS	+	+	+
Epicanthic folds	+	+	+
Clinodactyly of 5th fingers	+	+	+
Symphalangism of thumb	?	?	+
Hypothyroidism	?	+	−
Mental retardation	+	+	+
Pectus excavatum	?	+	−
Intrauterine leiomyoma	?	N/A	+

ring derivatives, in subsequent cell divisions [Hoo et al., 1974; Ledbetter et al., 1980; Côté et al., 1981]. It is possible that these ring derivatives may cause the rearrangement and/or interruption of other genes, including oncogene(s), which might be present on the particular chromosome involved.

We conclude that, in this fashion, activation of oncogene(s) within the 12p or 12q, and possibly a negative dominant effect, could be the underlying mechanism of leiomyoma formation in our patient. It could also be argued that a tumor suppressor gene is located in 12p or 12q, in which case loss of heterozygosity has augmented a germline mutation already present on the normal chromosome 12, thus initiating tumorigenesis.

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